Properties and Development of Valortim™ (MDX-1303): A potent, fully human mAb to PA for treatment of inhalational anthrax

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Medarex/PharmAthene Collaboration

- Valortim™ development began in October 2001
  - Collaborators: Dartmouth Medical School, USAMRIID
    - Identification of a fully human anti-PA mAb using Medarex UltiMAb HuMAb Development System®
  - Two NIAID/DMID grants awarded in September 04
    - Challenge grant – Product Development to FTIH
    - VTAD grant – MoA Studies & Therapeutic Models
- In November 2004, Medarex and PharmAthene entered into a collaborative agreement for advanced development and commercialization of Valortim™

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Valortim™: Key Features

- Selected on basis of superior performance in *in vitro* TNA
- Defines a novel neutralizing epitope on PA – NOT attachment
- Mechanism of action similar to protective immune response in vaccinated individuals – FcR dependence on activity
- Potent efficacy in prophylactic and also in therapeutic model of rabbit anthrax aerosol challenge
- Potent efficacy as a prophylactic in the model of monkey aerosol challenge
- Fully human IgGκ monoclonal antibody Medarex Ultimab
  - Produced in CHO cells in defined media
- No human safety issues observed in FTIH – fully enrolled
- Process development complete, Valortim™ ready for full scale production

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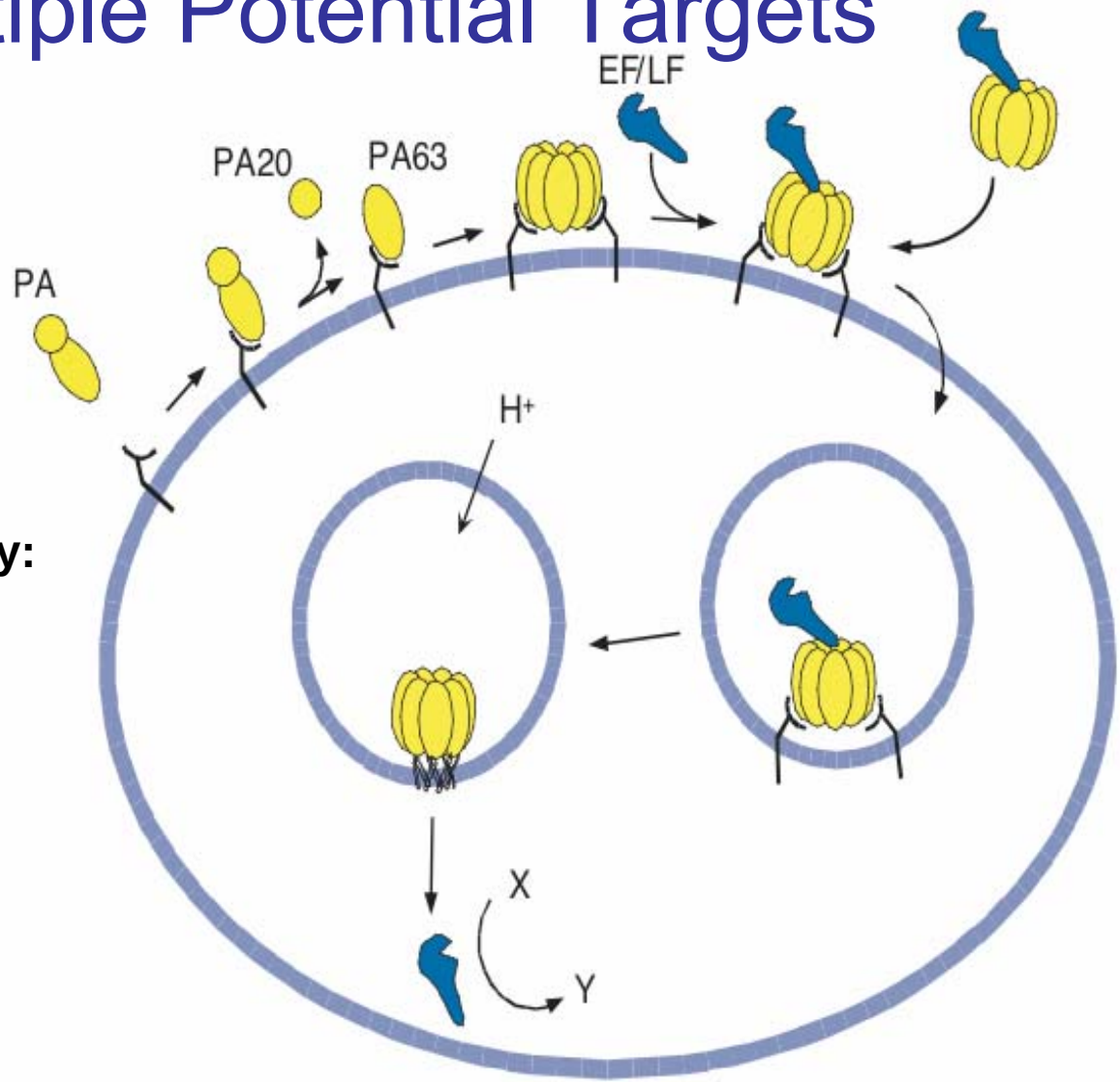
Antibodies to PA As Antitoxins: Multiple Potential Targets



Potential MAb Activity:

1. Attachment
2. Processing
3. Multimerization
4. Binding of EF/LF
5. Internalization
6. Pore formation

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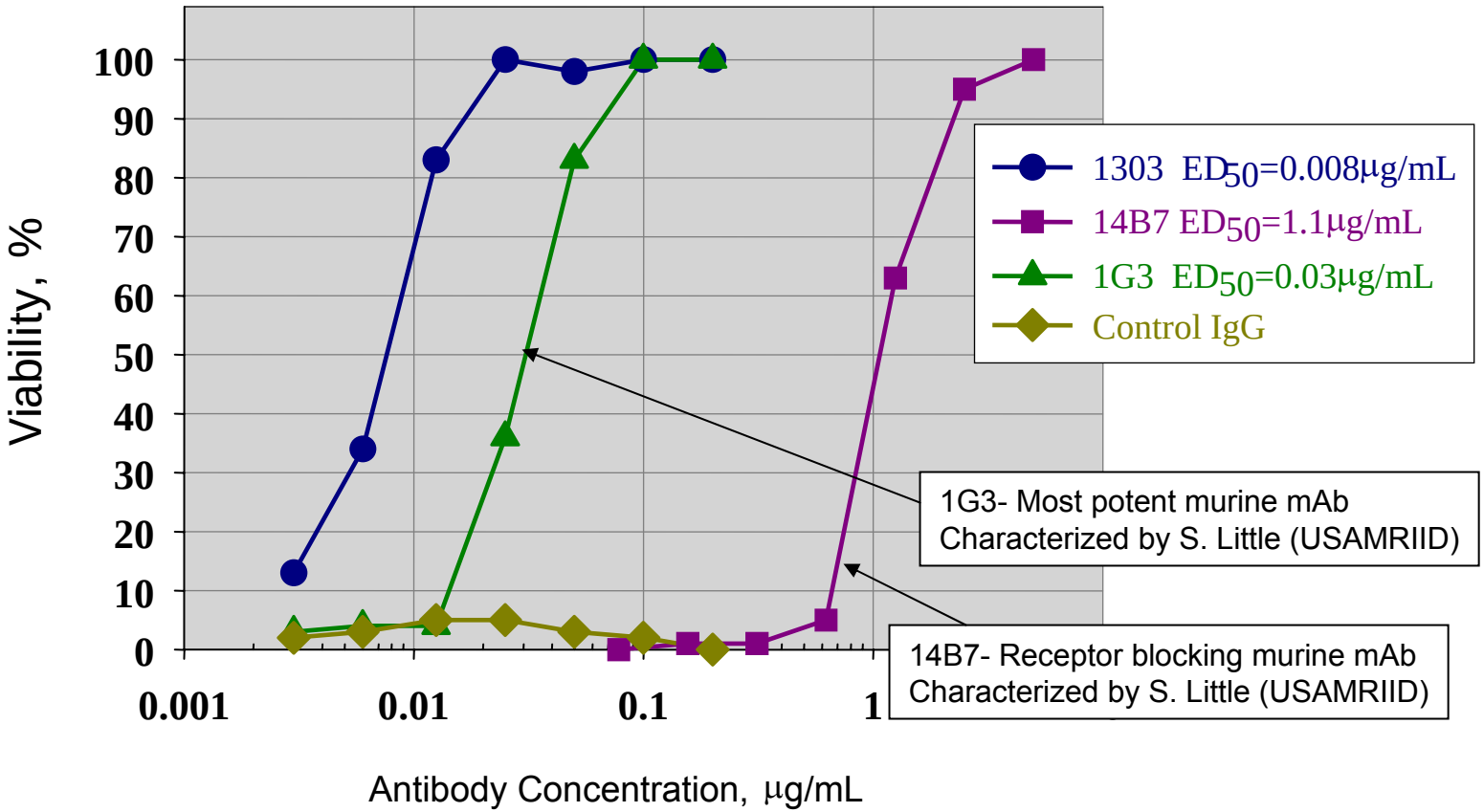
Assays for Lead MAb Selection?

- Affinity of Binding to PA-83/ PA-63
  - Highest affinity screening by Biacore, ELISA, etc…
- Blockade of Binding of PA to toxin receptor or to EF/LF
  - Anthrax Toxin Receptor: eg murine MAb 14B7
  - LF or EF component: eg murine MAb 1G3
- Potency in Toxin Neutralization Assay (TNA)
  - High titer immune sera correlate with TNA activity
  - Standard for vaccine response in clinical trials
- We chose to select HuMAbs with the best activity in TNA as leads, and figure out how they worked later

Valortim™ (MDX-1303): Toxin Neutralization Activity



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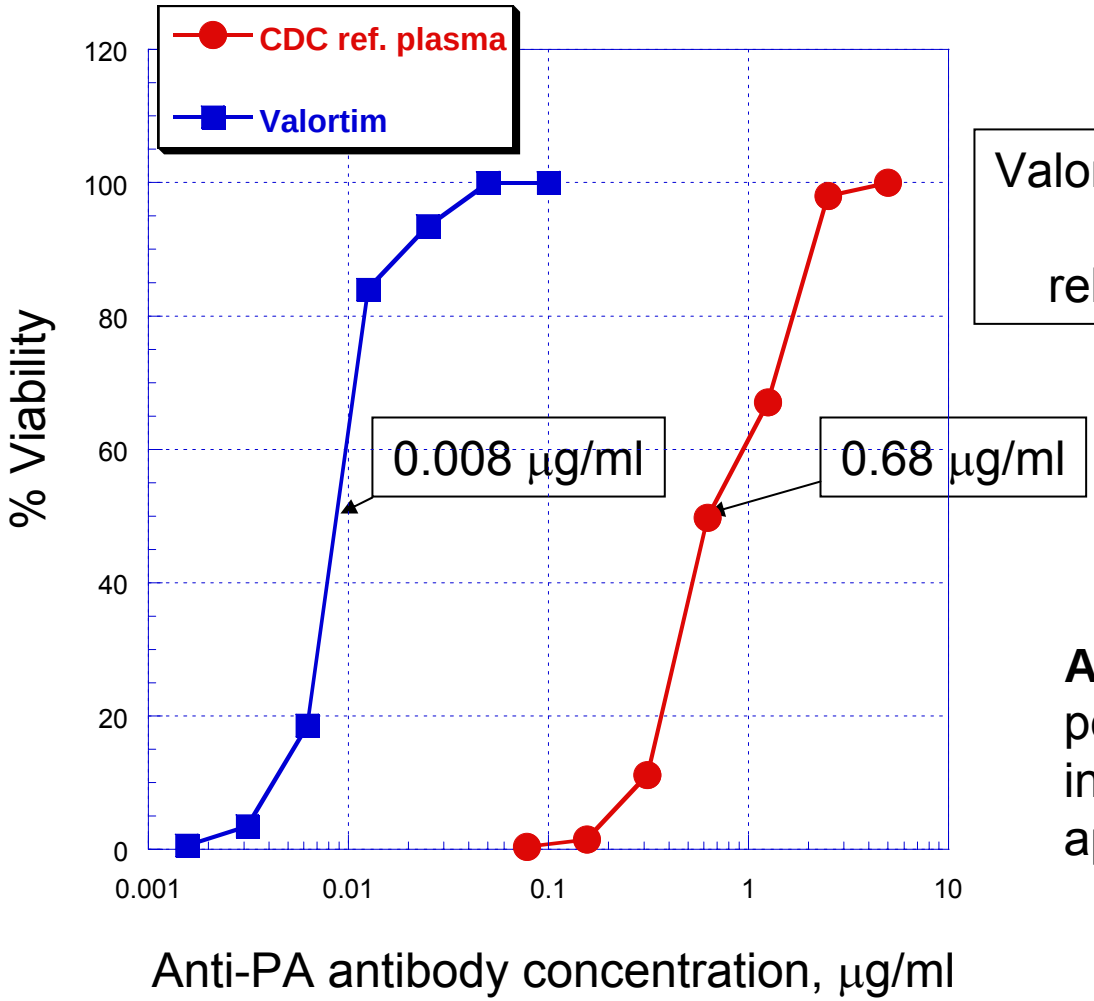
TNA activity of CDC human reference plasma

AVR801 = 109 μg/ml anti-PA IgG



Valortim: ~ 85x > specific activity

relative to AVR801

AVR801 Reference Standard: pooled polyclonal antisera from individuals vaccinated with approved AVA vaccine

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TNA Activity Relative to Other Mabs and to Human Reference Standard

Test Article	EC50(ng/ml)
Valortim™	8
1G3	30
AVR801*	680
14B7	1100

* CDC Human Reference Plasma

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CDC Evaluation of Valortim™ in TNA

Origin	ID	N	EC50 Mean (ng/ml)	StdDv	Proprietary EC50 (nM)	CDC EC50 (nM)
Medarex	Valortim™	24	6.39	1.77	0.13nM	0.04nM
Human Reference Standard	AVR414	25	131.48	34.17	Not Applicable	0.88nM

CDC Conclusions

Confirmed Valortim™ of high potency with an EC50 value of 6.39ng/ml (0.04nM)

Performance of Valortim™ has highly reproducible (SD of 1.77)

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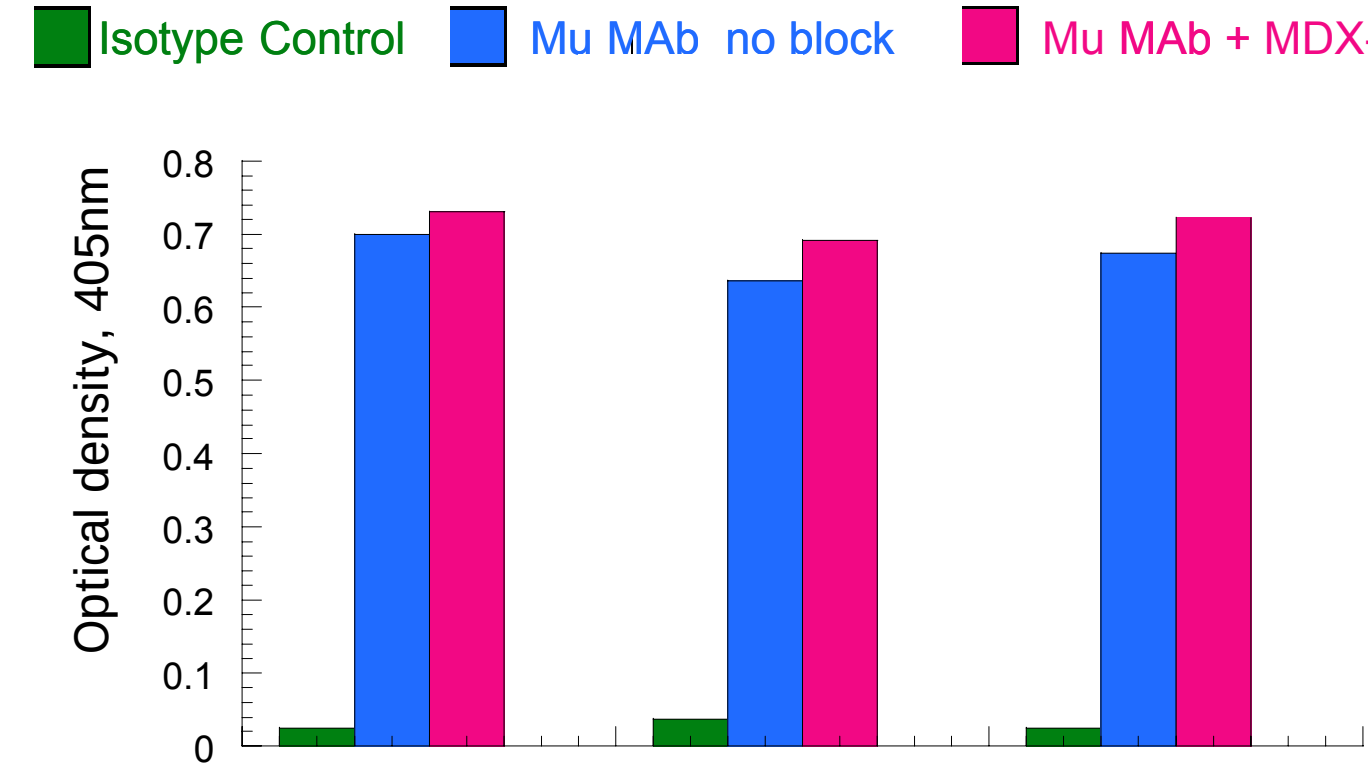
Valortim(MDX-1303) Defines a Novel Neutralizing Epitope



Mu Mab: 14B7 2D5 1G3

binding epitope : 671-721aa 581-601aa 168-314aa

Blocks receptor binding **Block LF binding**

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Little, et al Microbiology 1996,142:707-15

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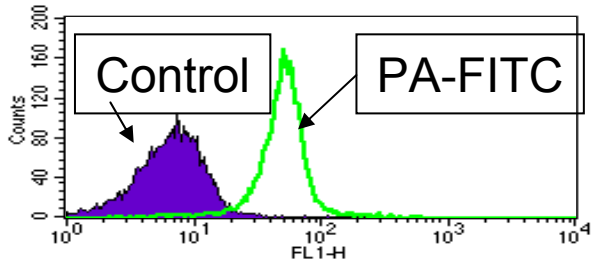
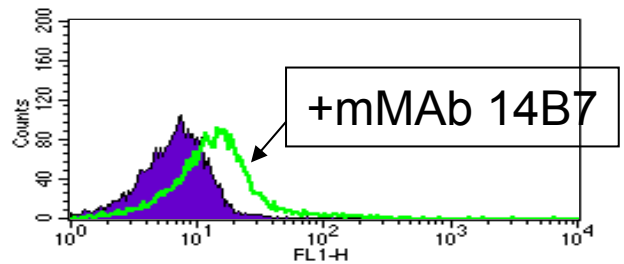
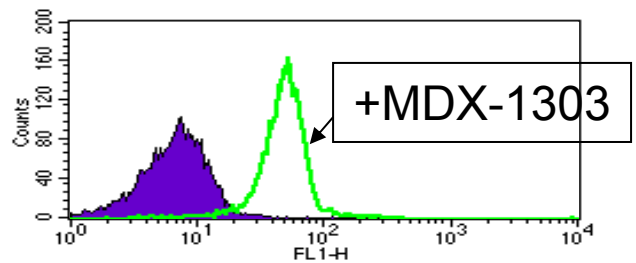
Receptor binding studies with human monocytes





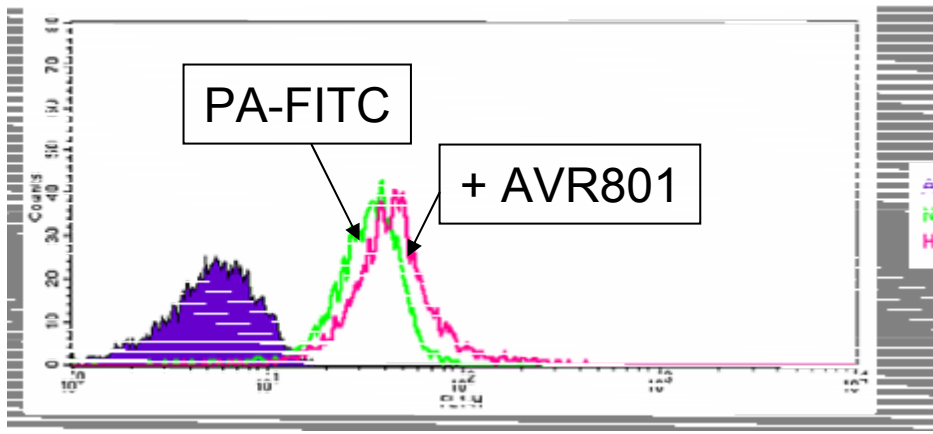
% blocking

78%



0 %

Valortim (MDX-1303) AND AVR801 do NOT block receptor binding!



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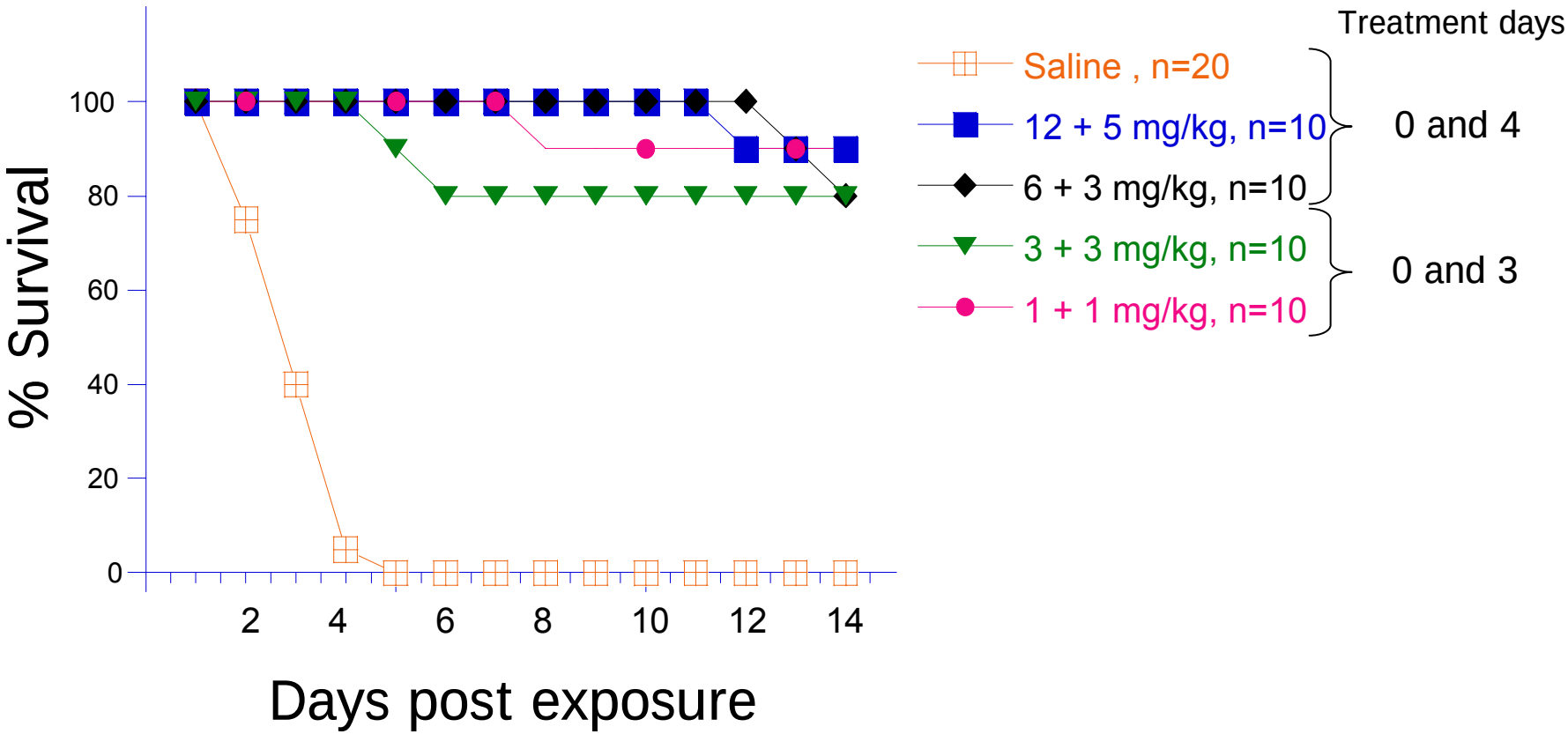
In Vivo Efficacy Data

Prophylactic Rabbit Inhalation Model: Dose Titration of Valortim (MDX-1303)



Exposure = 100-300 LD_{50}

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Valortim™ Rabbit Inhalation Model
Dose Response

Number of Rabbits	1 Hour Post Exposure	72 or 96 Hours Post Exposure	Survival
10	0 mg/kg	0 mg/kg	0/10
10	1 mg/kg	1 mg/kg	9/10
10	3 mg/kg	3 mg/kg	8/10
10	6 mg/kg	3 mg/kg	9/10
10	12 mg/kg	6 mg/kg	8/10

Lowest Effective Dose Not Yet Identified

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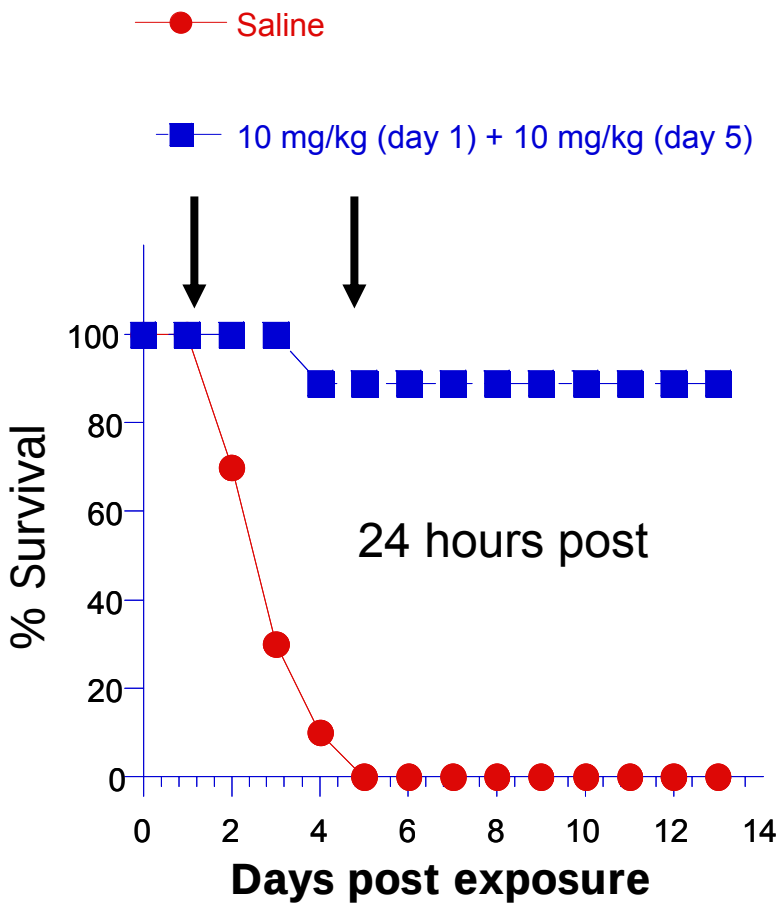
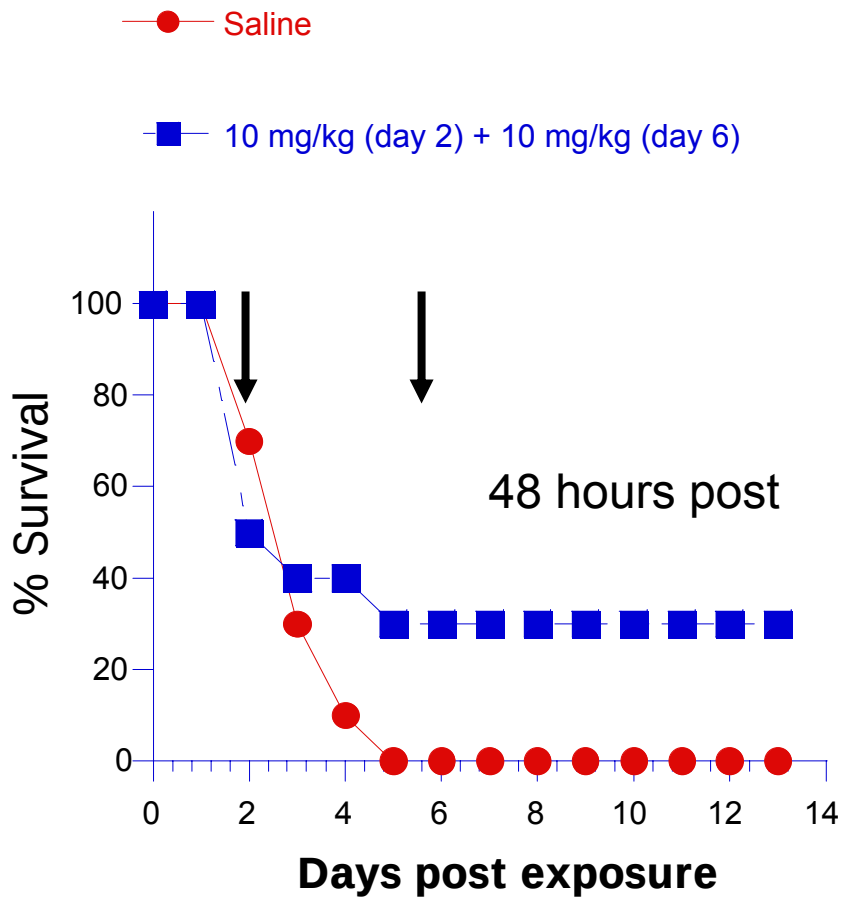
Rabbit Inhalation Model:
Delayed Treatment with Valortim (MDX-1303)





Exposure ~ 300 LD_{50}

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Valortim™ Rabbit Inhalation Model Delayed Treatment

Number of Rabbits	Antibody	Schedule of Treatments	Dose Level (mg/kg IV)		Survival
			1st	2nd	
10	Saline	1 hour post-exposure	0	0	0/10
10	Valortim™	24 hours post- exposure	10	10	8/9[a]
10	Valortim™	48 hours post-exposure	10	10	3/7[b]

Target LD50 = 200

[a]1 rabbit removed from study unrelated to anthrax or treatment

[b]3 rabbits died prior to treatment

Survival to day 14

Therapeutic Models are very challenging because of brittle process

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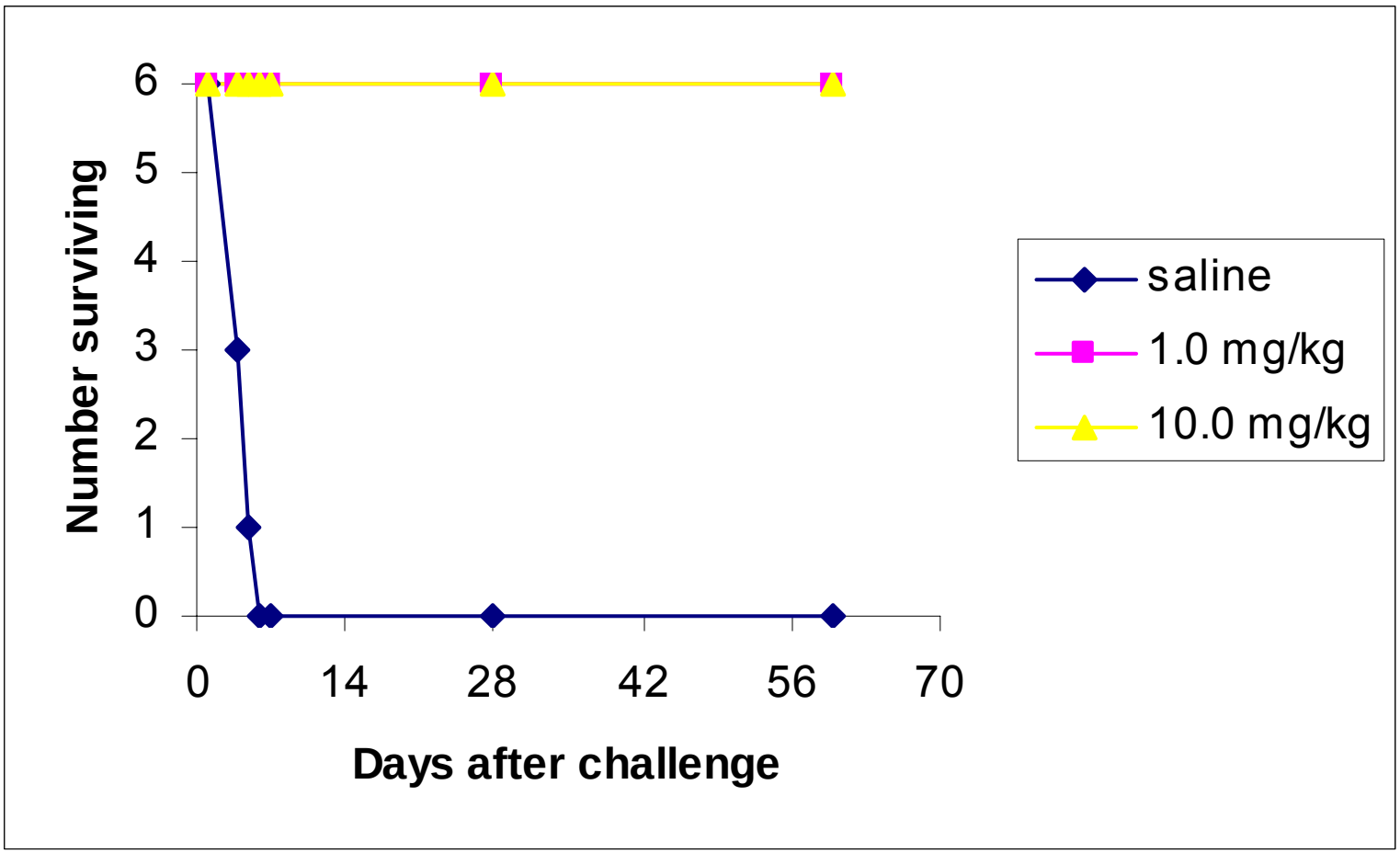
Cynomolgus Monkey Survival After Challenge and IM Injection of Valortim



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Valortim™ Monkey Inhalation Model

Number of Monkeys	Treatment	Dose Level 1 Hour Post Exposure	Survival
6	Saline	0 mg/kg IM	0/6
6	Valortim™	1 mg/kg IM	6/6
6	Valortim™	10 mg/kg IM	6/6

- Target LD50 = 200
- Survival to day 90 post-exposure
- Unprotected animals died within 4-7 days

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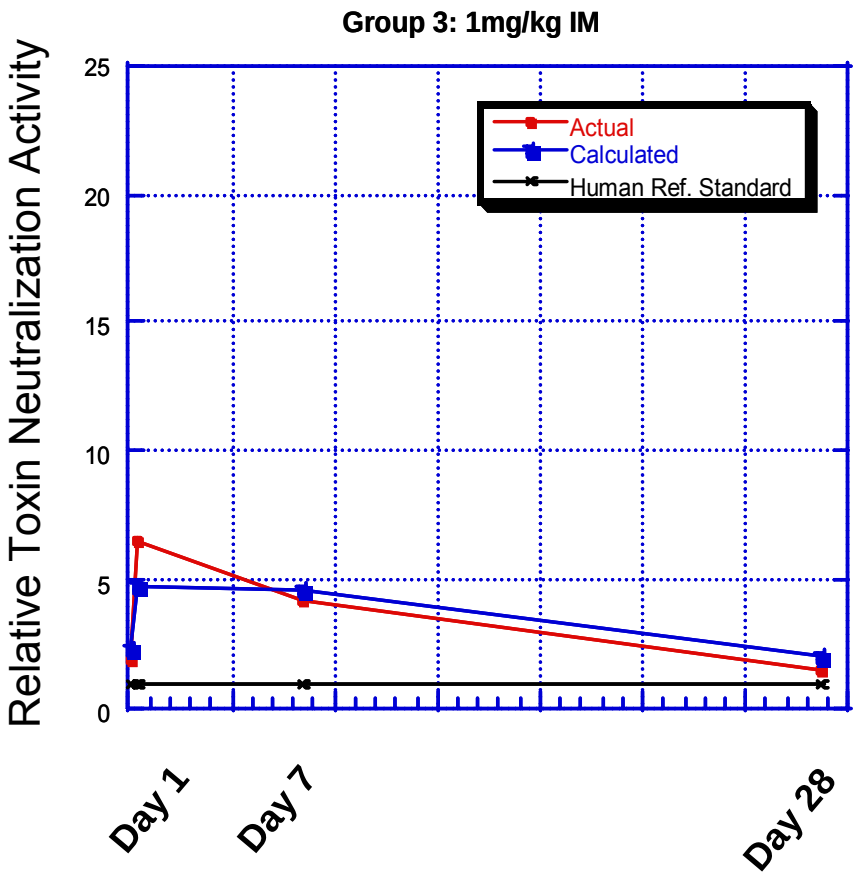
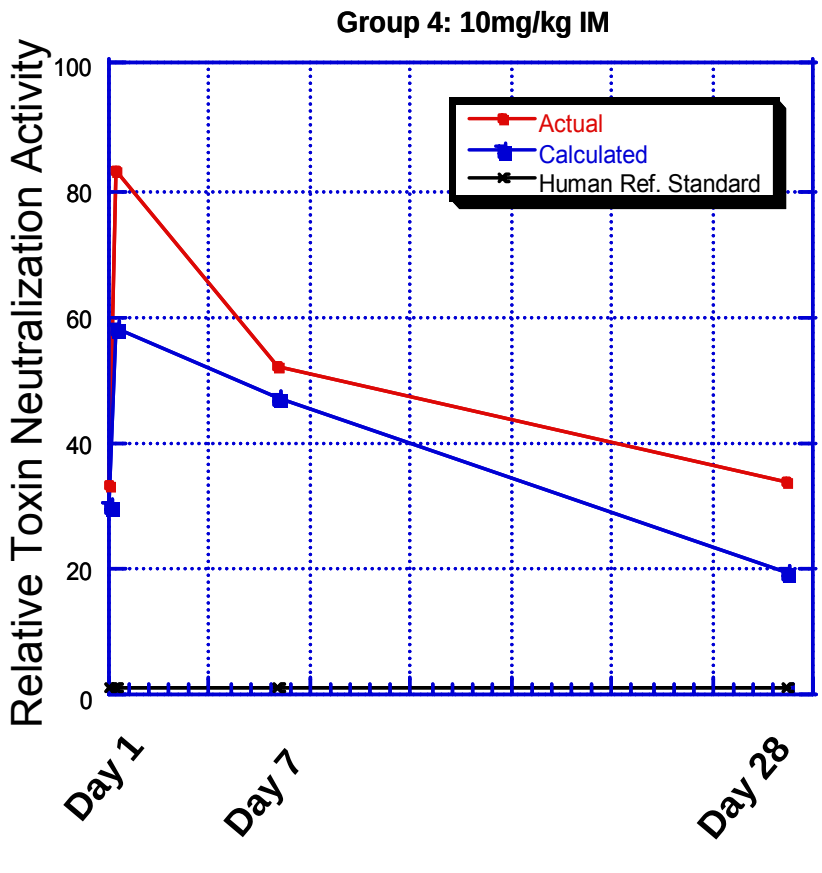
Valortim *in vivo* TNA PK profile

Calculated vs Actual TNA activity in uninfected Cynomolgus monkeys





CDC Human Reference Plasma- AVR801 , 109 μg/ml anti-PA IgG

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Valortim™ First Time in Human Study

Number of Subjects	Dose	Date of Dosing	Number of SAEs
3	0.3 mg/kg IV	28 Oct 05	0
10	1.0* mg/kg IV	29 Nov 05 30 Nov 05	0
10	3.0 mg/kg IV	05 Jan 06 06 Jan 06	0
10	10.0 mg/kg IV	02 Feb 06 03 Feb 06	0
3	20.0 mg/kg IV	02 Mar 06	0
10	100 * mg IM	08 Mar 06 09 Mar 06	0

*Doses of ≥ 1mg/kg predicted to be fully protective based on non-human primate studies.

Serum PK, TNA and immunogenicity analyses to commence soon

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Valortim - MDX-1303
Anti-PA HuMAb

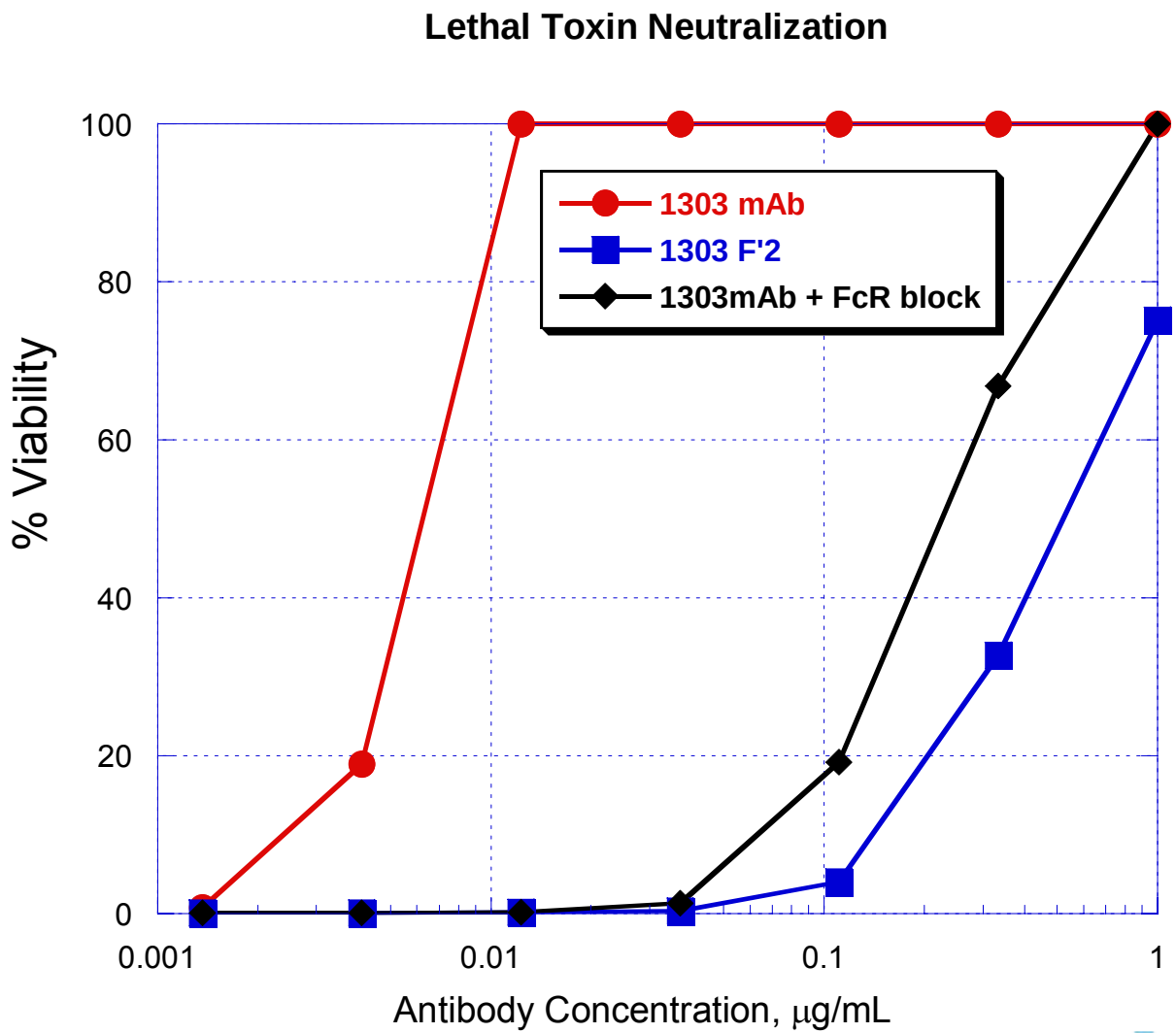
- Identified by functional screen: potent protection of J744A.1 cells from PA-LT complex mediated death (TNA)
- Potent in vitro neutralizing activity,
  - ED_{50} = 6-8 ng/ml, ED_{80} = 30 ng/ml (> than known murine MAbs)
  - High affinity to PA83& PA63, KD = 5.3 x 10^{-9} M
  - Novel epitope, near the receptor binding area but does not block
  - Can rescue macrophages after toxin complex exposure
- Potent in-vivo activity in rabbit AND monkey inhalation model:
  - Protects rabbits at 1 mg/kg (lowest tested to date)
  - Rescues symptomatic rabbits (3/7 survive at 48 hrs admin)
  - Fully protects non-human primates at dose of 1 mg/kg IM
  - After 28 days, 1 mg/kg IM in cyno TNA > CDC reference std
  - Does not block endogenous anti-anthrax immune response
- Well tolerated in normal volunteers at doses likely higher than needed

Mechanism of Action

- Optimal activity utilizes FcR binding

In Vitro Toxin Neutralization:

Valortim Activity is Markedly Enhanced by FcR Interaction



EC-50s:
6.5 ng/ml
500 ng/ml
220 ng/ml

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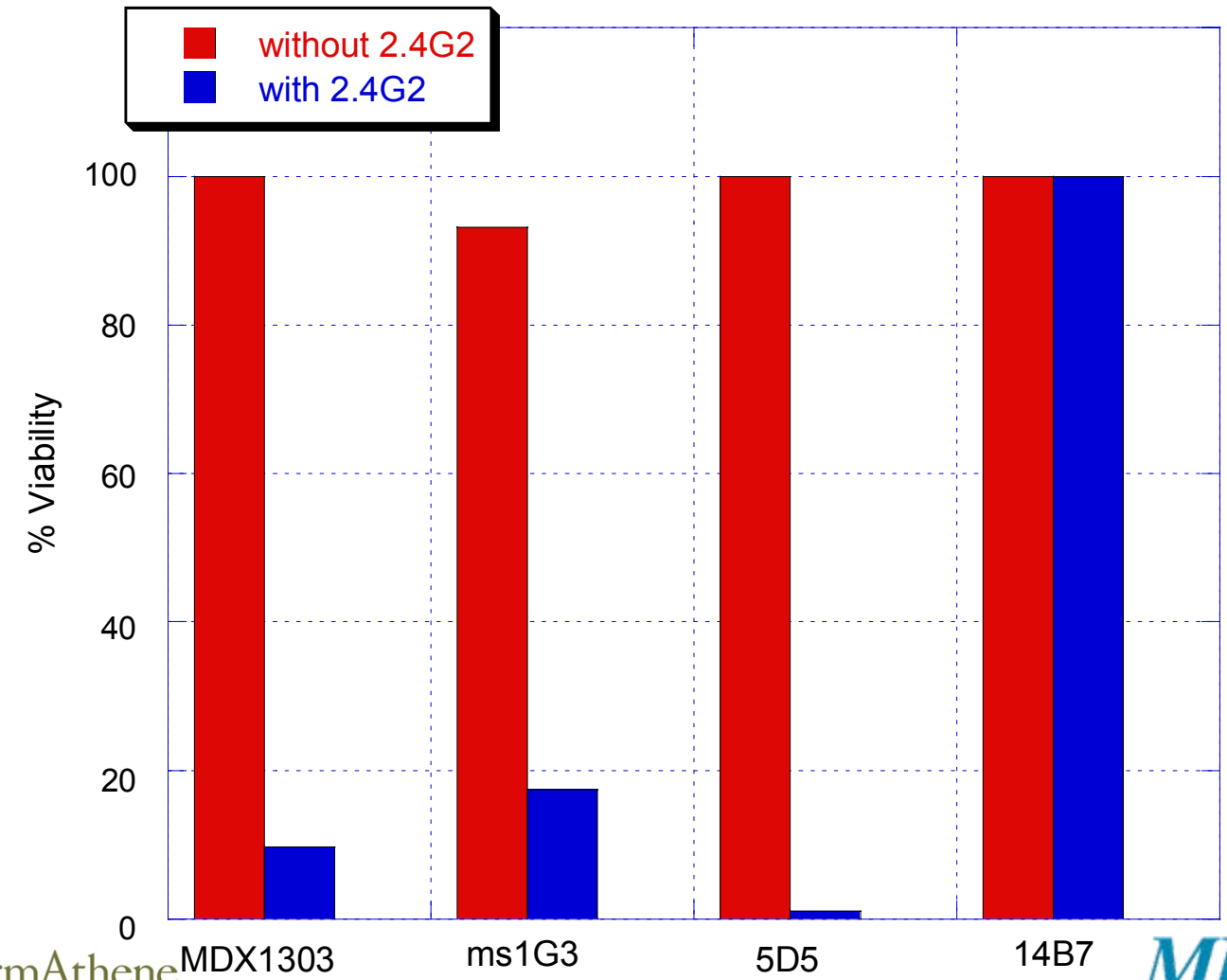
FcR Augmentation of Toxin Neutralization: Found in Some, But Not All MAbs



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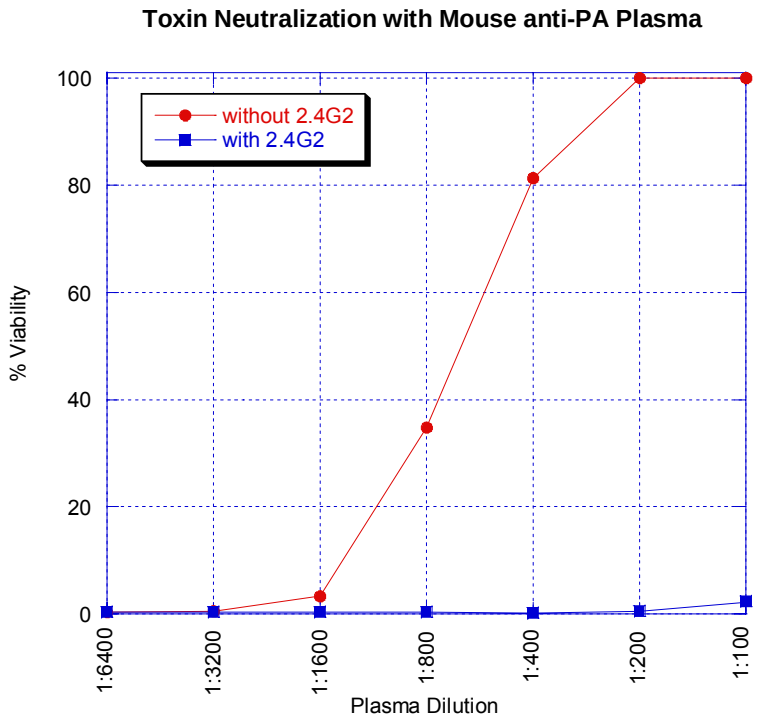
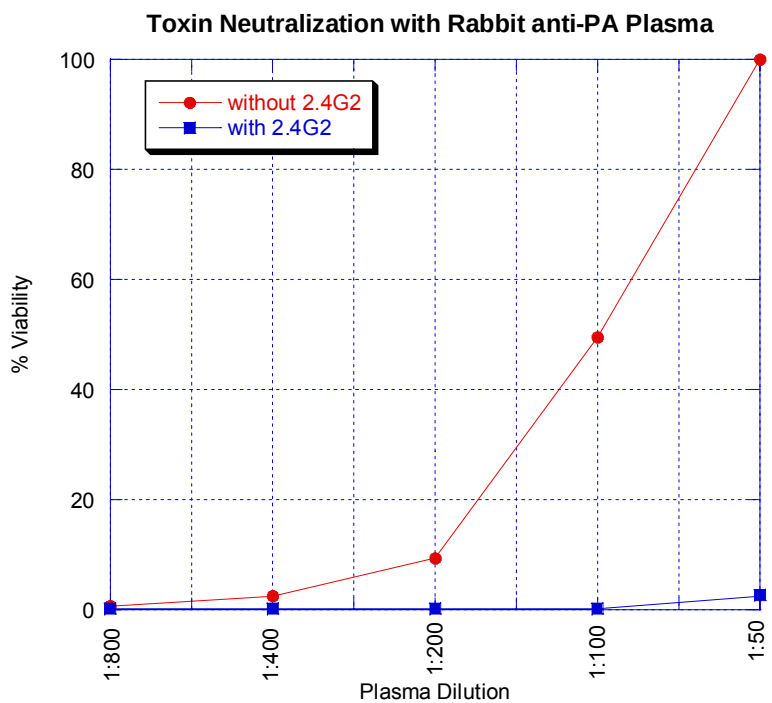
Toxin Neutralization by Polyclonal Anti-PA Sera: Markedly Augmented by FcR Interaction

Mouse



Rabbit



Anti-Fc receptor mAb 2.4G2 blocks in vitro TNA of anti-PA immune sera

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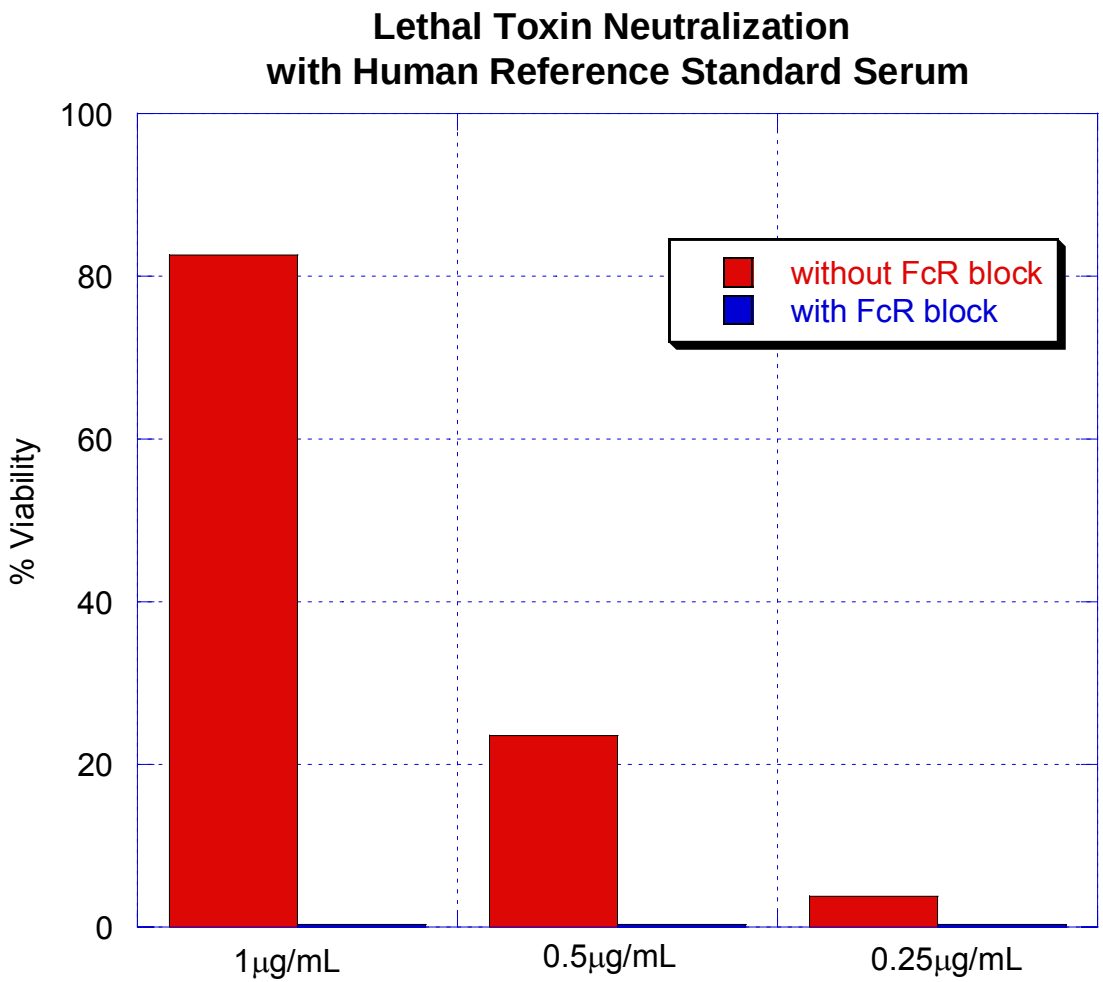
Human Toxin Neutralizing Antisera Elicited by Anthrax Vaccine Also Augmented by FcR Interaction



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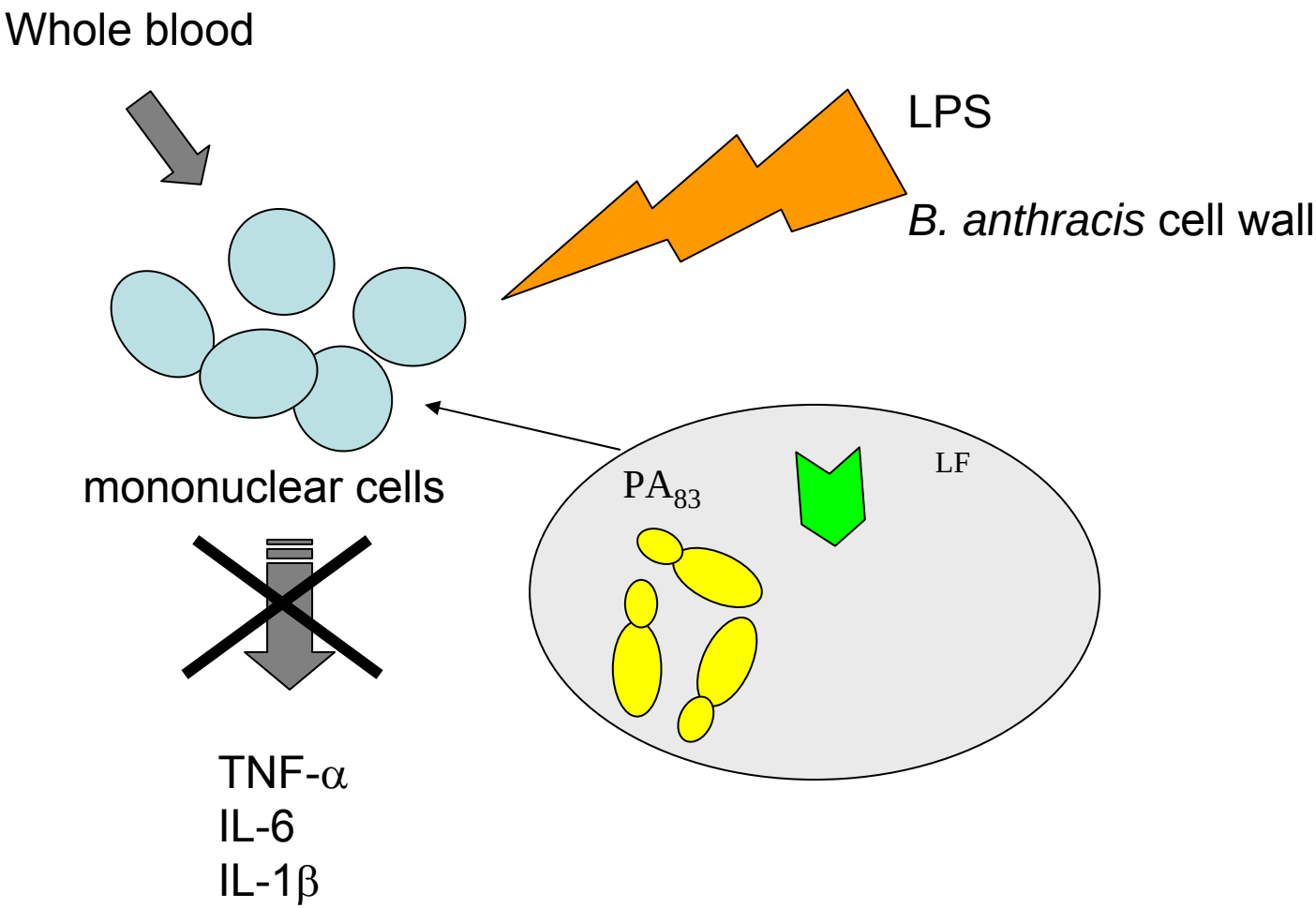
Human PBMC Cytokine Inhibition Assay
Alternative to Murine Macrophage Assay



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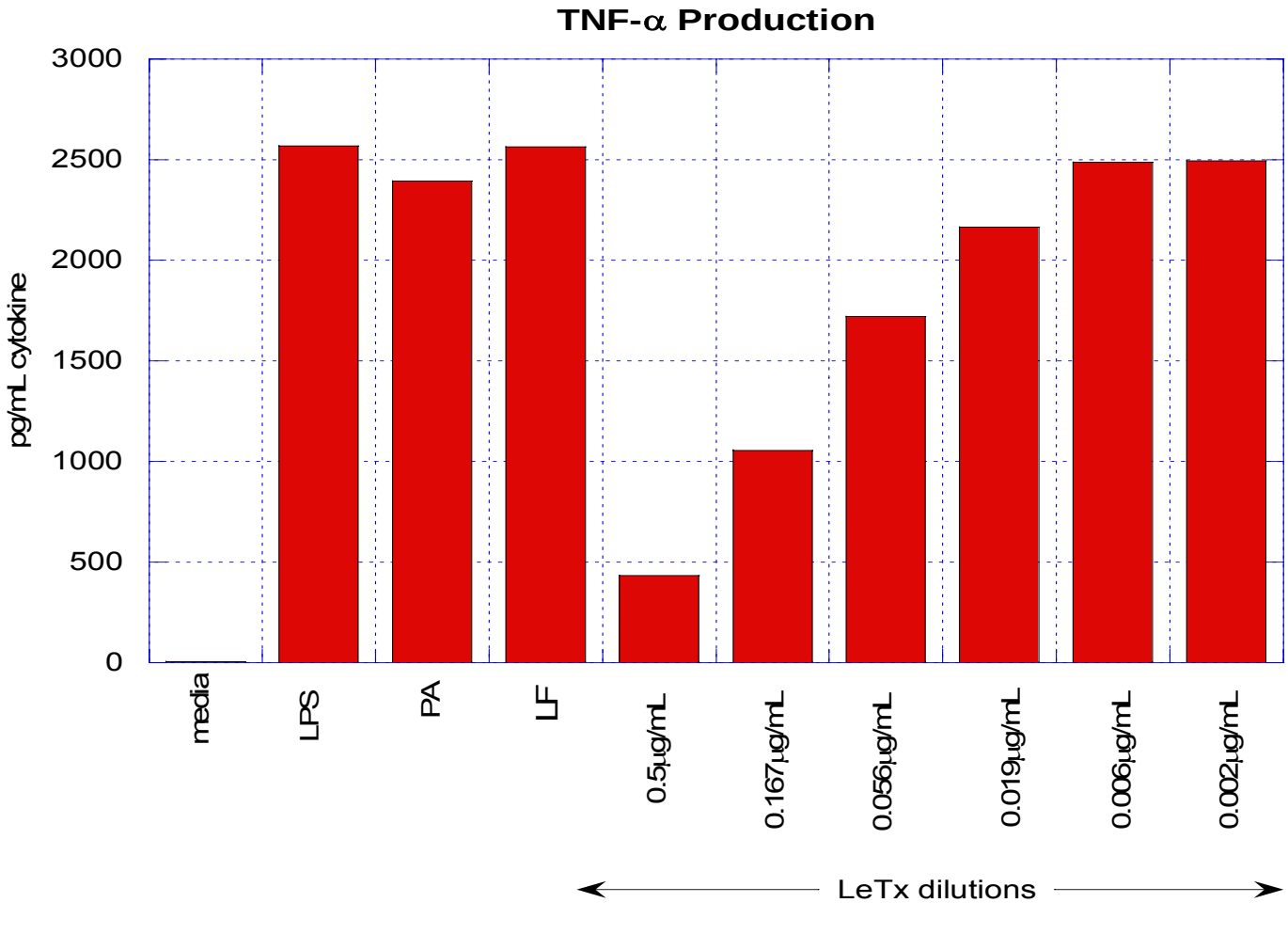
Human PBMC Cytokine Inhibition Assay
Toxin Dose Titration



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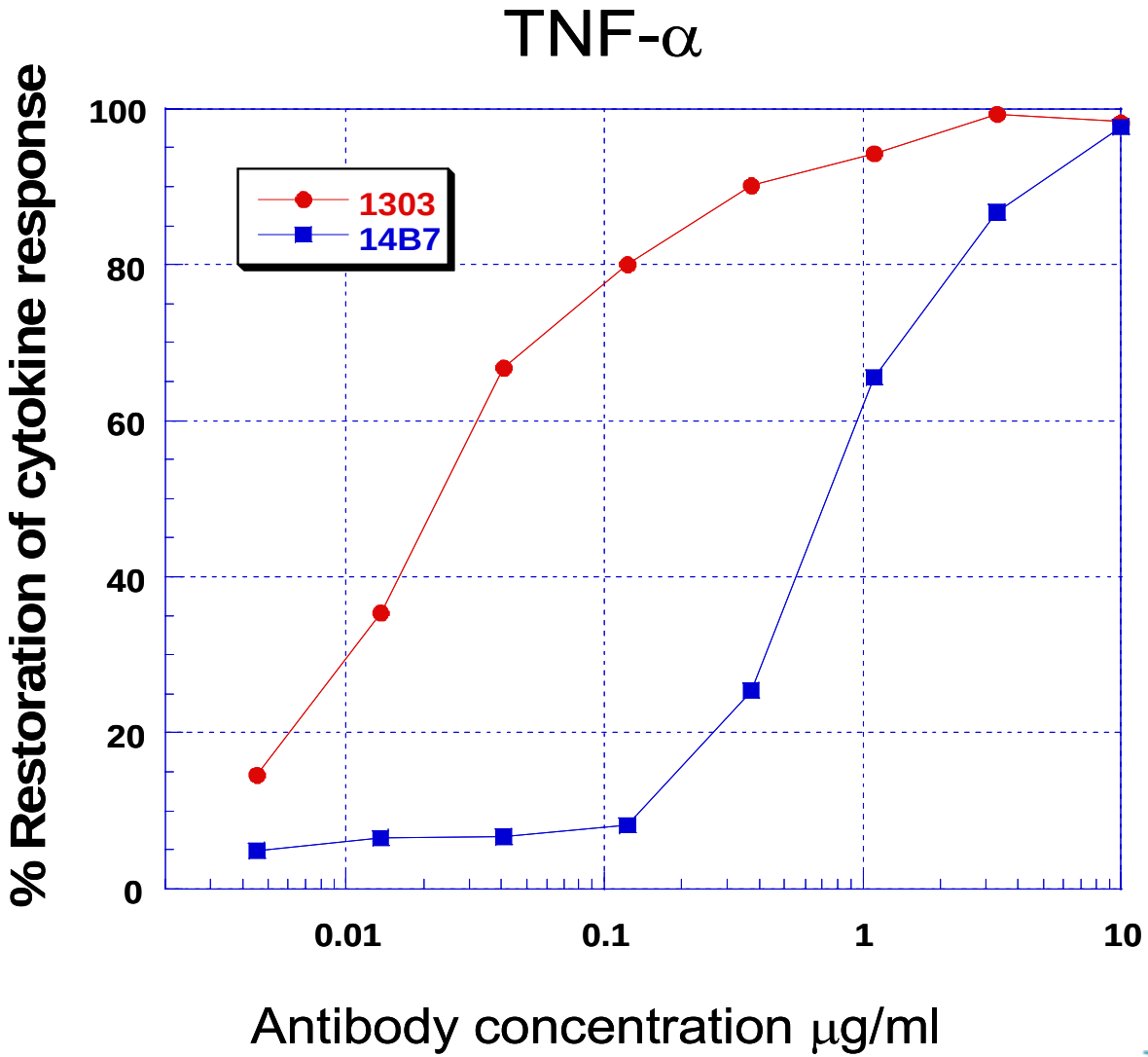
Human PBMC Cytokine Inhibition Assay
Valortim Potency



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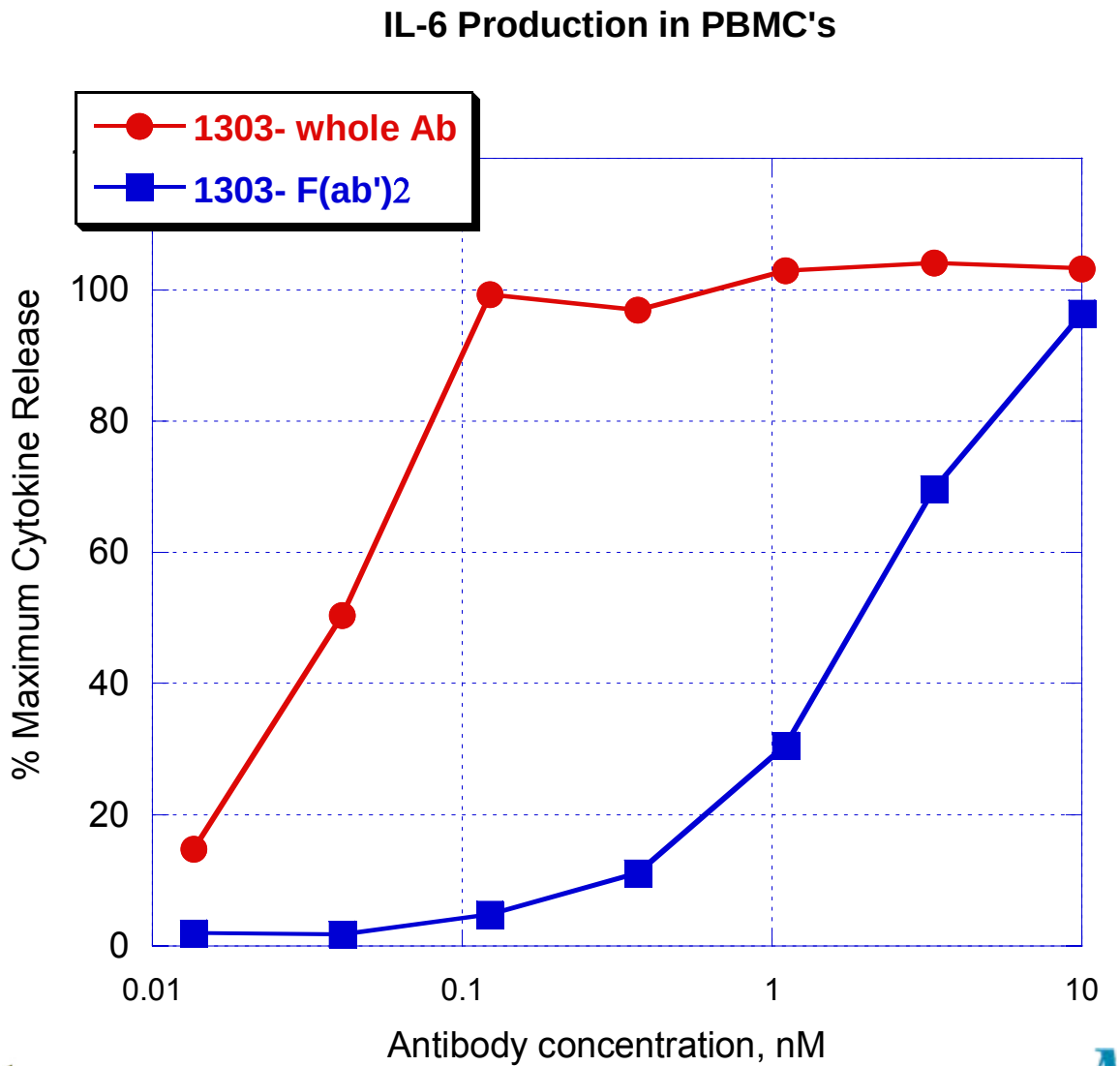
Human PBMC Cytokine Inhibition Assay
Potency Augmented by FcR Interaction



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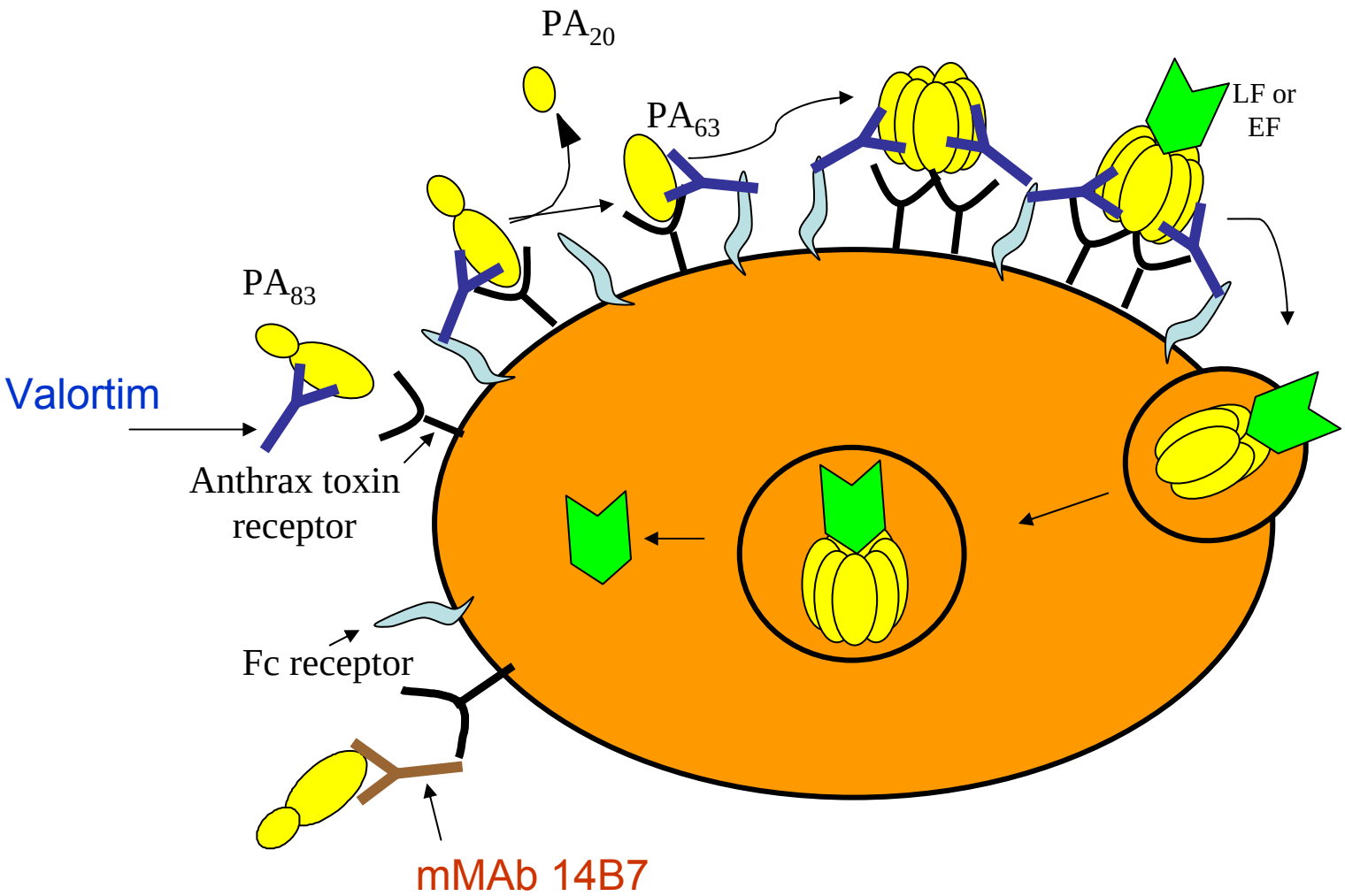
Anthrax Toxin and Proposed Mechanism of Neutralization by Valortim™



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Role of FcR Engagement *in vivo*

- Lowest effective dose of Valortim to date, 1 mg/kg still provides serum levels in excess of amounts that $F(ab)_2$ fragment would work – will lower amounts work?
- Notable that protective polyclonal immune responses in 3 species (mouse, rabbit and human) show:
  - Strong dependence on FcR interaction for activity in TNA, AND
  - Undetectable blocking activity for attachment (FcR independent)
  - Suggests FcR interaction is an important attribute of natural protection
- Definitive experiments for this role will require:
  - In vivo experiments with $F(ab)_2$ fragments
  - Experiments in animals with defined FcR mutations
  - Mutants of Valortim with FcR binding mutations

Summary

Valortim has an optimum profile for an anthrax anti-toxin

- Human monoclonal antibody
- High affinity and specificity
- Potent neutralizing activity in vitro and in vivo
- Mechanism of action same as vaccine induced immunity and distinct from other Mabs
- In vivo efficacy at doses as low as 1 mg/kg

Acknowledgements
Valortim Product Development

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